TOWNSEND
and
TOWNSEND
and
CREW
LLP

Denver, Colorado
Tel 303 571-4000

Palo Alto, California
Tel 650 326-2400

Seattle, Washington
Tel 206 467-9600

San Francisco

Two Embarcadero Center
Eighth Floor
San Francisco
California 94111-3834
Tel 415 576-0200
Fax 415 576-0300

May 7, 2004

VIA EXPRESS MAIL, WITH RETURN POSTCARD ENCLOSED

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
P.O. Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US2003/032086
Applicants: THE GOVERNMENT OF THE USA AS REPRESENTED BY THE SECRETARY OF THE DEPT. OF HEALTH AND HUMAN SERVICES et al.
Inventors: SHOEMAKER et al.
Filed: 08 October 2003
Express Mail Label No.: EV 383 385 199 US
Date of Mailing: 07 May 2004
Our File No.: 15280-4621PC

Dear Officer:

Enclosed are the Chapter II Demand and substitute page 24 of the specification submitted as an Amendment under Article 34. Enclosed also is a redlined version of the substitute page.

It is our belief that the above change does not include matter which go beyond the disclosure in the international application as filed.

Thank you for your attention to this matter.

Respectfully submitted,

Jean M. Lockyer, Ph.D.
Patent Agent

JML/elq

Enclosures: Chapter II Demand
Substitute page 24 of the Specification
Redlined Version of Substitute Page
Sequence Listing with Diskette
Transmittal Letter
Postcard

The demand must be filed directly with the competent International Preliminary Examining Authority; if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 15280-4621PC

International application No.	International filing date (*day/month/year*)	(Earliest) Priority date (*day/month/year*)
PCT/US2003/032086	08 October 2003 (08.10.03)	08 October 2002 (08.10.02)

Title of invention

IDENTIFICATION OF ANTI-HIV COMPOUNDS INHIBITING VIRUS ASSEMBLY AND BINDING OF NUCLEOCAPSID PROTEIN TO NUCLEIC ACID

Box No. II APPLICANT(S)

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

THE GOVERNMENT OF THE USA AS REPRESENTED BY THE SECRETARY OF THE DEPT. OF HEALTH AND HUMAN SERVICES
OTT-NIH
6011 Executive Boulevard, Room 325
Rockville, MD 20852
United States of America

Telephone No.: (301) 496-7056
Facsimile No.: (301) 402-0220
Teleprinter No.:
Applicant's registration No. with the Office: 44,879

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

SHOEMAKER, Robert H.
22606 Peach Tree Road
Boyds, MD 20841
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

CURRENS, Michael
615 Wilson Place
Frederick, MD 21702
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

☒ Further applicants are indicated on a continuation sheet.

International application No.
PCT/US2003/032086

Continuation of Box No. II **APPLICANT(S)**

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

REIN, Alan
7295 Swan Point Way
Columbia, MD 21045
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

FENG, Ya-Xiong
10300 West Lake Drive, Apt. 305
Bethesda, MD 20817
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

FISHER, Robert
17408 Miller's Sawmill Road
Sharpsburg, MD 21782
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

STEPHEN, Andrew
114 North Beechwood Avenue
Catonsville, MD 21228
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

☒ Further applicants are indicated on another continuation sheet.

International application No.
PCT/US20037032086

Continuation of Box No. II **APPLICANT(S)**

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

WORTHY, Karen
13801 Wanegarden Drive
Germantown, MD 20874
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

SEI, Shizuko
10104 Galsworthy Place
Bethesda, MD 20814
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
JP	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

CRISE, Bruce
208 Chestnut Avenue
Washington Grove, MD 20880
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

HENDERSON, Louis E.
10296 Quail Trail
Mt. Airy, MD 21771
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/US2003/032086

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LOCKYER, Jean M.
TOWNSEND AND TOWNSEND AND CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
United States of America

Telephone No.: 415-576-0200

Facsimile No.: 415-576-0300

Teleprinter No.:

Agent's registration No. with the Office: 44,879

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of**:

☒ the international application as originally filed

the description ☐ as originally filed
☒ as amended under Article 34

the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☒ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of applicable time limit under Rule 69.1(d).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

International application No.
PCT/US2003/032086

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

				For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:	1	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter	:	1	sheets	☐	☐
6. other (*specify*)	:		sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate power of attorney
3. ☐ original general power of attorney
4. ☐ copy of general power of attorney; reference number, if any: ;
5. ☐ statement explaining lack of signature
6. ☒ sequence listing in computer readable form
7. ☐ tables in computer readable form related to a sequence listing
8. ☒ other (*specify*): Transmittal Letter
   Postcard

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

X _____

Jean M. Lockyer
TOWNSEND AND TOWNSEND AND CREW LLP
USPTO Reg. No.: 44,879
Applicants' Agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.
   ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.
6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54*bis*.1(a) and item 7 or 8, below, does not apply
7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54*bis*.1(a) as extended by virtue of Rule 80.5.
8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54*bis*.1(a), the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand

For International Preliminary Examining Authority use only

International application No.	PCT/US2003/032086
Applicant's or agent's file reference	15280-4621PC

Date stamp of the IPEA

Applicant

THE GOVERNMENT OF THE USA AS REPRESENTED BY THE SECRETARY OF THE DEPT. OF HEALTH AND HUMAN SERVICES et al.

CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee	600.00	P
2. Handling fee (*Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.*)	162.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	762.00 TOTAL	

MODE OF PAYMENT

- ☒ authorization to charge deposit account with the IPEA (see below)
- ☐ cash
- ☐ cheque
- ☐ revenue stamps
- ☐ postal money order
- ☐ coupons
- ☐ bank draft
- ☐ other (*specify*):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT
(*This mode of payment may not be available at all IPEAs*)

- ☒ Authorization to charge the total fees indicated above.
- ☒ (*this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit*) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

IPEA/ US

Deposit Account No.: 20-1430

Date: 07 May 2004

Name: Jean M. Lockyer

Signature:

See Notes to the fee calculation sheet

High-throughput screen

[0084] A high throughput screen was used to detect small molecules that disrupted the association of viral NC-p7 protein with an oligonucleotide. The assay was performed using a Tomtec Quadra robotic system. NC protein was immobilized on Costar (Corning, NY) high-bind polystyrene 96 well plates by incubating 100 µl 250nM NC-p7 in phosphate buffered saline (PBS)/ 10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween overnight at 4°C. 200µl of 2% BSA in PBS/10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween was added and incubated at room temperature for 1-2 hours in order to block the plates. The plates were then washed 2 times with 200µl PBS/10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween using a Titertek M96V plate washer and stored overnight at 4°C. 5nM biotinylated 28 base oligonucleotide (5' GACTTGTGGAAAATCTCTAGC AGTGCAT 3'; SEQ ID NO:1) in PBS/10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween was added to each well followed by 10µM of test compound (in 20% dimethyl sulphoxide) from the Diversity Set and allowed to incubate at room temperature for 1 hour. Plates were then washed 3 times with 200µl PBS/0.05% Tween. Binding of the biotinylated 28mer was measured by adding 100µl 1:20000 dilution of nutravidin-horse radish peroxidase (stock 0.8mg/ml, from Pierce Chemical Co. Rockford, Il) and incubating at room temperature for 1 hour. The plates were washed 3 times with 200µl PBS/0.05% Tween. Plates were developed by adding 100µl of Supersignal (Pierce Chemical Co. Rockford, Il) and bioluminescence was measured in a Wallac Victor plate reader. Each plate had eight positive (5mM EDTA in PBS) and eight negative (20% DMSO alone) controls. The hit threshold was set at 100% inhibition. Active compounds that were identified included NSC 13778, NSC 13746, NSC 13755, and NSC 28620.

Secondary screen

[0085] A secondary screen was used to identify small molecules that disrupt *in vitro* assembly of Gag precursor protein into virus-like particles. Artificial viral particles were assembled from recombinant Gag protein and tRNA as previously described (*see, e.g.,* Campbell &Rein, "In vitro assembly properties of human immunodeficiency virus type 1 Gag protein lacking the p6 domain" *J. Virol.* 73: 2270-2279, 1999).

[0086] Two series of active compounds were identified that disrupted viral assembly. One series, an aromatic antimony-containing series, is based on the antimony-containing

High-throughput screen

[0084] A high throughput screen was used to detect small molecules that disrupted the association of viral NC-p7 protein with an oligonucleotide. The assay was performed using a Tomtec Quadra robotic system. NC protein was immobilized on Costar (Corning, NY) high-bind polystyrene 96 well plates by incubating 100 µl 250nM NC-p7 in phosphate buffered saline (PBS)/ 10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween overnight at 4°C. 200µl of 2% BSA in PBS/10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween was added and incubated at room temperature for 1-2 hours in order to block the plates. The plates were then washed 2 times with 200µl PBS/10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween using a Titertek M96V plate washer and stored overnight at 4°C. 5nM biotinylated 28 base oligonucleotide (5' GACTTGTGGAAAATCTCTAGC AGTGCAT 3') (SEQ ID NO:1) in PBS/10µM zinc chloride/10mM β-mercaptoethanol/0.05% Tween was added to each well followed by 10µM of test compound (in 20% dimethyl sulphoxide) from the Diversity Set and allowed to incubate at room temperature for 1 hour. Plates were then washed 3 times with 200µl PBS/0.05% Tween. Binding of the biotinylated 28mer was measured by adding 100µl 1:20000 dilution of nutravidin-horse radish peroxidase (stock 0.8mg/ml, from Pierce Chemical Co. Rockford, Il) and incubating at room temperature for 1 hour. The plates were washed 3 times with 200µl PBS/0.05% Tween. Plates were developed by adding 100µl of Supersignal (Pierce Chemical Co. Rockford, Il) and bioluminescence was measured in a Wallac Victor plate reader. Each plate had eight positive (5mM EDTA in PBS) and eight negative (20% DMSO alone) controls. The hit threshold was set at 100% inhibition. Active compounds that were identified included NSC 13778, NSC 13746, NSC 13755, and NSC 28620.

Secondary screen

[0085] A secondary screen was used to identify small molecules that disrupt *in vitro* assembly of Gag precursor protein into virus-like particles. Artificial viral particles were assembled from recombinant Gag protein and tRNA as previously described (*see, e.g.,* Campbell &Rein, "In vitro assembly properties of human immunodeficiency virus type 1 Gag protein lacking the p6 domain" *J. Virol.* 73: 2270-2279, 1999).

[0086] Two series of active compounds were identified that disrupted viral assembly. One series, an aromatic antimony-containing series, is based on the antimony-containing

OWNSEND
and
OWNSEND
and
CREW
LLP

Palo Alto
Tel 650 326-24

Denver, Colorado
Tel 303 571-4000

Walnut Creek, California
Tel 925 472-5000

Seattle, Washington
Tel 206 467-9600

San Francisco
Two Embarcadero Center
8th Floor
San Francisco
California 94111-3834
Tel 415 576-0200
Fax 415 576-0300

May 7, 2004

VIA EXPRESS MAIL

Special Programs Examiner Sue Wolski
Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US2003/032086
filed October 8, 2003
Applicant(s): The Government of the United States of America *et al.*
Inventor(s): Shoemaker *et al.*
Title: Identification of Anti-HIV Compounds Inhibiting Virus Assembly and Binding of Nucleocapsid Protein to Nucleic Acid
Our Ref.: 15280-4621PC

Dear Special Programs Examiner Wolski:

In order to comply with the WIPO Standard ST.25 requirements for the Sequence Listing, please find enclosed the Sequence Listing and disk at this time.

It is hereby stated that "the information recorded on the computer readable form is identical to the written sequence listing" and does not include matter which goes beyond the disclosure in the international application as filed.

Sincerely,

Jean M. Lockyer, Ph.D.
Patent Agent

JML:dmw
Enclosures
60209915 v1

SEQUENCE LISTING

<110> Shoemaker, Robert H.
Currens, Michael
Rein, Alan
Feng, Ya-Xiong
Fisher, Robert
Stephen, Andrew
Worthy, Karen
Sei, Shizuko
Crise, Bruce
Henderson, Louis E.
The Government of the United States of America
as represented by The Secretary of the
Department of Health and Human Services

<120> Identification of Anti-HIV Compounds Inhibiting Virus Assembly and Binding of Nucleocapsid Protein to Nucleic Acid

<130> 015280-462100PC

<140> WO PCT/US03/32086
<141> 2003-10-08

<150> US 60/416,854
<151> 2002-10-08

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:biotinylated 28 base oligonucleotide for viral NC-p7 protein association assay

<400> 1
gacttgtgga aaatctctag cagtgcat 28